Prayogik Rasayan

Evaluation of antiurolithiatic potential in *Crataeva religiosa:* An *in vitro* study

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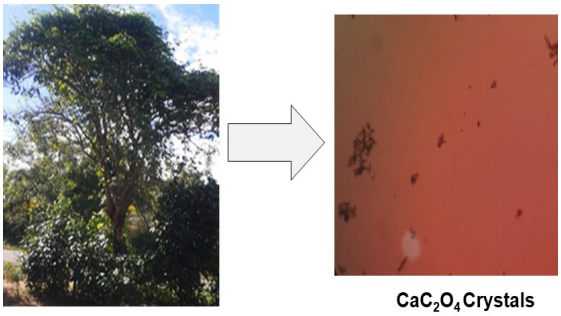
Received: February 3, 2019 | Accepted: February 23, 2019 | Published online April 17, 2019

Abstract

Urolithiasis is the condition where urinary calculi are formed in the urinary tract. Although the recurrence of urinary calculi formation could not be prevented with modern medicine, the plant species used in folklore medicine could serve as potential sources of novel antiurolithiatic agents. *Crataeva religiosa* is a plant employed in indigenous medicine in Sri Lanka to dissolve urinary calculi and to treat other urinary disease conditions. Thus, herein, the antiurolithiatic potential of methanolic extract of bark of *C. religiosa* was studied under *in vitro* conditions using crystal nucleation, aggregation and dissolution assays. The *in vitro* assays revealed that the above extract is capable of preventing crystal nucleation and aggregation as well as dissolving the urinary calculi. Its efficacy was comparable with cystone, a marketed polyherbal combination and it indicated that the bark of *C. religiosa* was a potent and promising antiurolithiatic agent, in accordance with its use in traditional medicine. However, further investigations on its chemical profile and possible cytotoxic effects are required before developing it as a therapeutic agent.



Crataeva religiosa

Keywords: antiurolithiatic, *Crataeva religiosa*, herbal, urinary calculi

1. Introduction

The deposition or formation of urinary calculi in any part of the urinary system i.e. the kidney, the ureters or the urinary bladder is called as urolithiasis[1]. It is one of the most prevalent diseases affecting nearly 4-15% of the human population worldwide[2]. A calculi is an aggregation of solute materials from urine such as calcium, oxalate, phosphate and uric acid which forms solid concretion[3]. Calcium oxalate has been identified as the predominant component of the majority of calculi formed in the urinary system.

The modern techniques available in the management of urinary calculi are extracorporeal lithotripsy, ureteroscopy and local calculus disruption using a high power laser[4]. However, drawbacks such as traumatic effects of shock waves, persistent residual stone fragments and recurrence of stone formation along with a number of other side effects associated with these therapeutic approaches could not be neglected[3]. In that sense, search for new antilithiatic drugs from natural sources such as medicinal plants has assumed greater importance, as herbal drugs are believed to be cost effective and with low side effect profiles. Plant extracts may contain phytochemicals capable of inhibiting the synthesis and aggregation of crystals as well as with a potency to remove or dissolve the already existing calculi[5].

A number of plant species are employed in traditional systems of medicine in Sri Lanka to treat and/or prevent urinary calculi. For example, *Crataeva religiosa* (Family: Capparaceae) is renowned as a remedy for various urinary complaints and specially, a decoction of the powdered bark is widely utilized to cure calculi in the kidney or bladder[6]. Thus the present study is undertaken to evaluate the *in vitro* antiurolithiatic potential in *C. religiosa* and thereby to scientifically validate its folklore claims.

2. Methodology

2.1. Preparation of plant extract

Bark of *C. religiosa* was collected from Gampaha district in Western Province of Sri Lanka in 2018 and authenticated from the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (WP2018-NO_09) was deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka. The

plant materials were air dried, cut into small pieces and extracted in methanol for two days. Thereafter, the liquid aliquot obtained by filtration was evaporated into complete dryness and six test concentrations (1000, 500, 250, 125 and 62.5 µg/mL) were prepared using this crude extract.

2.2 Determination of antiurolithiatic activity

2.2.1 Nucleation assay

The inhibitory activity of the extracts on the nucleation of CaC_2O_4 crystals was determined by a spectrophotometric assay based on the method described by Hennequin et al,[7] with slight modifications. Crystallisation was initiated by adding $CaCl_2$ (4 mmol/L) and $Na_2C_2O_4$ (50 mmol/L) solutions to artificial urine in the presence of plant extract at different concentrations. The nucleation was determined by the appearance of crystals that reached a critical size and became optically detectable in the presence of the extract and without the extract (negative control). The absorbance was recorded at 620 nm, and the percentage inhibition was calculated as [(C- S)/C] x 100, where, C is the turbidity without plant extract, S is the turbidity with plant extract. Cystone was used as the positive control.

2.2.2 Aggregation assay

The rate of aggregation of the CaC_2O_4 crystals was determined by the method of Hess et al,[8] with slight modifications. The CaC_2O_4 crystals were prepared by mixing both the solutions of $CaCl_2$ and $Na_2C_2O_4$ at 50 mmol/L which were equilibrated in a bath for 1 h at 60 °C. The solutions were cooled to 37 °C and then evaporated. The CaC_2O_4 crystals were dissolved with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5 to a final concentration of 1 mg/mL. The absorbance at 620 nm was recorded at 30, 60, 90, 180 and 360 min. The percentage inhibition was calculated as [(C-S)/C] x 100, where, C is the turbidity without plant extract, S is the turbidity with plant extract. Cystone was used as the positive control.

2.2.3 Dissolution of urinary calculi

Surgically removed urinary calculi were obtained from patients admitted to District General Hospital, Gampaha and Teaching Hospital Karapitiya, Sri Lanka. These calculi were crushed to homogenize the sample and were (weight range 30-90 mg) put into each test tube and different concentrations of extracts were added. These were allowed 24, 48, and 72 hours for dissolution at 37±0.5℃ and the weight of the calculi were obtained at 24, 48, 72 hour intervals. After the dissolution period, the reduction in weight and percentage weight loss were calculated using following formula.

% Weight loss = $[(W_i - W_f)/W_i] \times 100$ where W_i is the initial weight of the calculi and W_f is the final weight of the calculi

Cystone was used as positive control while distilled water was used as negative control.

2.3 Statistical analysis

All experiments were conducted in duplicates and statistical analysis of the data was performed by Analysis of Variance (ANOVA). A probability value of difference p~0.05 was considered as statistically significant. All data were presented as mean values ± standard deviation (SD).

2.4 Ethical consideration

Ethical approval was obtained from the Ethical Review Committee, Faculty of Allied Health Sciences, University of Ruhuna.

3. Results

3.1 Nucleation assay

As the calculi formation begins with the occurrence of nuclei, the classical model for the study of oxalate crystallization was employed in this study. As depicted in Fig 1 and 2, the inhibition of nucleation was concentration dependent and the maximum inhibition percentage was observed at the concentration of 2000 µg/mL. At this concentration, the plant extract inhibited the calcium oxalate crystal nucleation by 15.1% while 17.4% inhibition was observed for cystone, the positive control employed in this study.

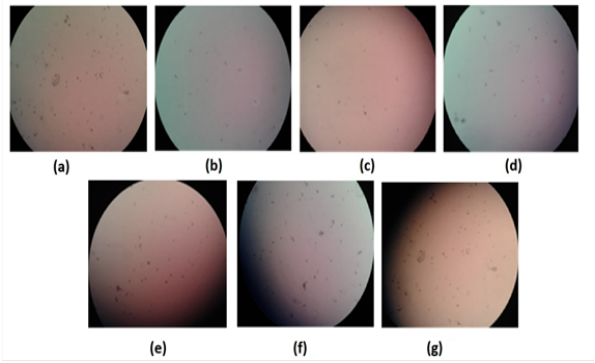


Fig.1 Microscopic evaluation of *C. religiosa* extract on CaC_2O_4 nucleation (40 × magnification) (a) Negative control (without the extract) (b) 2000 µg/mL (c) 1000 µg/mL (d) 500 µg/mL (e) 250 µg/mL (f) 125 µg/mL (g) 62.5 µg/mL.

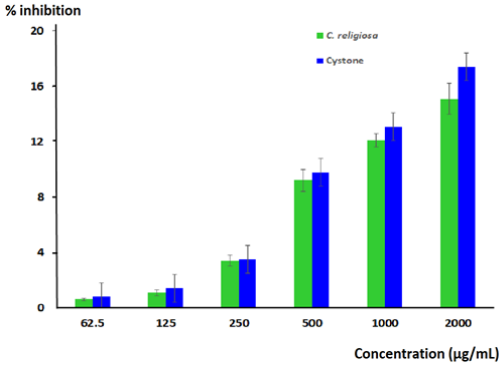


Fig.2 Effect of *C. religiosa* extract and cystone on CaC_2O_4 nucleation at different concentrations

3.2 Aggregation assay

Urinary crystals tend to binds with one another through a process known as aggregation/agglomeration. These adhered crystals are held in place, thus cannot be easily separated and

as a result, it plays an important role in lithiasis. Therefore, the inhibitory potential of the plant extract on crystal aggregation was studied and compared with the commercial polyherbal remedy. The results revealed that the plant extract at its highest concentration (2000 µg/mL) could effectively inhibit the crystal aggregation and this potency is comparable with that of the positive control (Fig.4). Further, the inhibition of aggregation was not found to be time dependent.

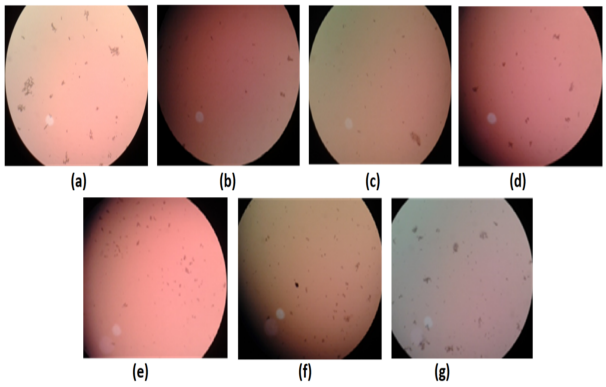


Fig.3 Microscopic evaluation of *C. religiosa* extract on CaC_2O_4 crystal aggregation (40 × magnification). (a) Negative control (without the extract) (b) 2000 µg/mL (c) 1000 µg/mL (d) 500 µg/mL (e) 250 µg/mL (f) 125 µg/mL (g) 62.5 µg/mL.

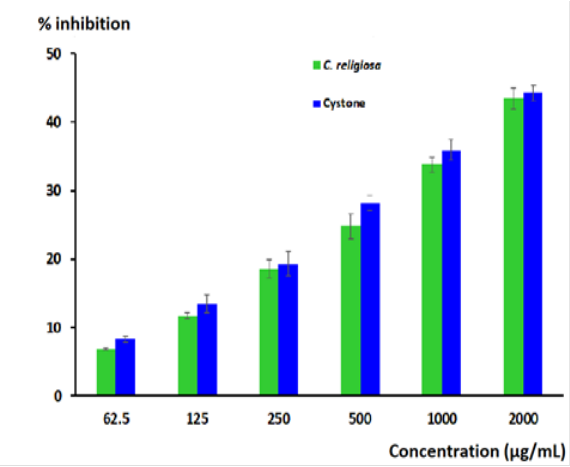


Fig.4 Effect of *C. religiosa* extract and cystone on CaC_2O_4 aggregation at different concentrations

3.3 Dissolution of urinary crystals

The dissolution of urinary calculi was expressed as percentage weight change at different time intervals. The percentage weight loss with the plant extract was found to be directly proportional to the increase in the concentration of the extract where maximum mean percentage weight loss (4.32%) was observed at 2000 µg/mL concentration. Moreover, the percentage weight loss was found to be time dependent and the highest mean percentage weight loss of urinary calculi was observed after 72 hours. However, the highest activity for cystone was observed at 500 µg/mL (4.27%), and the activity of the plant extract at 2000 µg/mL and cystone at 500 µg/mL was not found to be statistically significant.

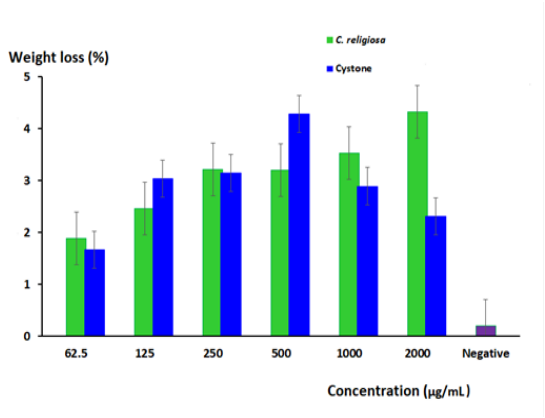


Fig.4 Effect of *C. religiosa* extract and cystone on dissolution of urinary calculi at different concentrations after incubation of 72 hours

4. Discussion

Formation of urinary calculi is a complex process that results from a succession of several physicochemical events including super saturation, nucleation, nucleus growth, nucleus aggregation, and retention within renal system[2]. In many cases, the calculi are very small and can pass out of the body without any problems. However, even a small calculi could block the flow of urine resulting excruciating pain and prompt medical treatment may be needed under these circumstances[9]. Although many remedies have been used to remove and dissolve the urinary calculi, high incidence of adverse effects associated with these approaches has motivated humans to return to nature for the search of non/less toxic alternatives. Thus the present investigation was undertaken to investigate the antiurolithiatic activity of *C. religiosa* which is renowned as a herbal remedy for urinary disorders in traditional Sri Lankan medicine. The *in vitro* investigations revealed that *C. religiosa* is highly effective as an antiurolithiatic agent and this could be attributable to the presence of antiurolithiatic phytochemicals in this extract. Thus, experiments will be conducted to identify the bioactive secondary metabolites while detailed *in vivo* assays and cytotoxicity studies are planned for the scientific validation of its efficacy and safety and thereby to develop it as an alternative therapy for urinary calculi.

5. Conclusion

Our investigations clearly indicated that under *in vitro* conditions, methanolic extract of bark of *C. religiosa* has a potent antiurolithiatic activity due to its inhibitory effects on the crystal nucleation, aggregation as well as dissolution of urinary calculi. However, further studies are necessary to isolate and characterize the compounds responsible for antiurolithiatic activity and to evaluate possible cytotoxic effects in *in vitro* and *in vivo* models, in order to assess the suitability of this extract to develop as an antiurolithiatic agent at commercial scale.

6. Acknowledgements

MN gratefully acknowledge India-Sri Lanka Joint Research Project-2017 for the financial assistance to conduct research work on medicinal plants; Department of Biochemistry, Faculty of Medicine and Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences for providing laboratory facilities to conduct this study.

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